MALARIA SPECIES AND SOUTHEAST ASIAN OVALOCYTOSIS DEFINED BY A 27-BP DELETION IN THE ERYTHROCYTE BAND 3 GENE

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Abstract. To evaluate the resistance of SAO against species specific malaria infection, relationships between parasite species and the 27-bp deletion in the band 3 gene were studied in malaria endemic Sumba Island, eastern Indonesia. Thick blood films were prepared from patients with malaria symptoms (n=129) and healthy controls (n=231). Species of *Plasmodium* was identified by microscopic observation. The 27-bp deletion was screened by the PCR method. Among 231 healthy controls, 29 (12.6%) had the 27-bp deletion, whereas 14 (10.9%) among 129 patients confirmed with malaria infection harbored the 27-bp deletion. No significant difference was observed in the prevalence of the 27-bp deletion between controls and patients ($p>0.8$). There was no significant difference in the frequency of the 27-bp deletion between *P. vivax* and *P. falciparum* infected subjects at 5% level by Fisher's exact test. The present result showing no correlation between the presence of the 27-bp deletion and infected parasite species is consistent with the post-invasion resistance hypothesis that may involve not a single malaria species.

INTRODUCTION

Microscopic observation of peripheral blood films has revealed a wide distribution of Southeast Asian/Melanesian ovalocytosis (SAO) in parts of Southeast Asia and Melanesia (Lieinjo, 1976; Amato and Booth, 1977). The underlying molecular defect of SAO involves heterozygous presence of a 27-bp deletion in the band 3 gene, which causes a 9-amino acids deletion and consequently functional defect of the band 3 proteins on the erythrocyte membrane (Jarolim *et al*, 1991; Tanner *et al*, 1991). By epidemiological and experimental studies, a hypothesis that SAO is resistant to malaria was highlighted (Serjeantson *et al*, 1977; Mohandas *et al*, 1984; Foo *et al*, 1992). However, it is still unclear whether the resistance of SAO to malaria is limited to a single malaria species or effective for multiple species. There were studies concluding that SAO is much more resistant to *Plasmodium vivax* than *Plasmodium falciparum* and vise versa (Serjeantson *et al*, 1977; Foo *et al*, 1992).

To evaluate the resistance of SAO against species specific malaria infection, we studied relationships between parasite species and the 27-bp deletion in malaria endemic Sumba Island, eastern Indonesia.

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MATERIAL AND METHOD

To simplify the ethnic frame of the subjects, the ethnic Sumba were selected. Thick blood films were prepared from healthy controls (n=231) and patients with malaria symptoms (n=129) after oral informed consent was given. After microscopic diagnosis of *Plasmodium* species, DNAs were extracted from the thick blood films and the 27-bp deletion was screened by the PCR method (Jarolim *et al*, 1991).

RESULT AND DISCUSSION

Among 231 healthy control subjects, 29 (12.6%) had the 27-bp deletion, whereas among 129 malaria parasite infected individuals with symptoms, 14 (10.9%) harbored the deletion (Table 1). There was no significant difference in the prevalence of the 27-bp deletion between controls and patients ($p>0.8$). We classified the parasite carriers into three categories: (1) with *P. falciparum*, (2) with *P. vivax*, and (3) with both species. Between *P. vivax* and *P. falciparum* infected subjects, there was no significant difference in the frequency of the 27-bp deletion at 5%level by Fisher's exact test (Table 2).

Table 1
Category of the subjects studied.

	Number of		
Band 3 gene	Controls	Patients	Total
With del	29	14	43
Without del	202	115	317
Total	231	129	360

del: the 27-bp deletion in the band 3 gene.

Table 2
The 27-bp deletion in the band 3 gene and *Plasmodium* species.

	Number of subjects with			
Band 3 gene	*Pf*	*Pv*	*Pf* & *Pv*	Total
With del	6	5	3	14
Without del	69	21	25	115
Total	75	26	28	129

Pf: *Plasmodium falciparum.*
Pv: *Plasmodium vivax.*
del: the 27-bp deletion in the band 3 gene.

The frequency of the 27-bp deletion was not significantly different between *Pf* and *Pv* infected subjects at 5% level by Fisher's exact test ($p=0.085$ and 0.107 for *Pf* and *Pv*, respectively).

The result shows that the presence of the 27-bp deletion in the band 3 gene did not alter the incidence of malarial disease by *P. falciparum* and *P. vivax*. It is thus concluded that the resistance of the molecularly defined SAO to malaria infection does not differ by the parasite species. We have surveyed for the 27-bp deletion among Southeast Asian ethnic groups who had been shown to carry morphologically defined SAO in the literature and found unexpectedly low frequencies of the 27-bp deletion among them (Kimura *et al*, 1998), while we have encountered microscopically indistinguishable ovalocytic erythrocytes without the 27-bp deletion (Kimura *et al*, submitted). Moreover, SAO with recessive inheritance (Amato and Booth, 1977) and SAO without the 27-bp deletion were reported (Tanner *et al*, 1991). Controversial implications of the relationship between parasite species and SAO in the earlier studies might be due to an admixture of morphologically defined SAO caused by different molecular bases.

The resistance of SAO to malaria used to be assigned to the rigid erythrocyte membrane that might interfere with parasite invasion (Kidson *et al*, 1981; Mohandas *et al*, 1992). However, an *in vitro* study using erythyrocytes of SAO with the 27-bp deletion did not prove the resistance during the parasite invasion (Dluzeuski *et al*, 1992). It was suggested that post-invasion factors such as a rapid decline in erythrocyte ATP concentration play roles in the resistance. Another observation, which also supported the post-invasion resistance of SAO to malaria was reported by a hospital based study (Genton *et al*, 1995); none of the subjects with the 27-bp deletion developed severe cerebral malaria. This brought an idea that the mutated band 3 protein reduces cytoadherence of parasitized erythrocytes to cerebral microvessels and conduces to less severe symptoms. Since parasite invasion into erythrocytes is mediated by the species-specific receptors, our present result showing no correlation between the presence of the 27-bp deletion and infected parasite species is consistent with the post-invasion resistance hypothesis.

As well documented by Miller (1994),

certain genetic polymorphisms have been selected for by malaria. SAO in the Oriental World probably corresponds to the sickle cell disease in Africa but not to the Duffy null; the former interferes with parasite growth in a post-invasion manner, and the latter serves as a perfect barrier only against *P. vivax* invasion. The mechanism of the malaria resistance attained by the 27-bp deletion is still not fully understood and remains to be elucidated.

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